Determination Effect of Common Used Insecticide as Dichlorvous, Diazinon and Cypermethrin *in vitro* in the Camel

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Abstract

Using modified electrometric method to validate the measurement of plasma and erythrocyte cholinesterase activities camel. At several temperature degrees 37.5 lead to given an optimum enzymatic reaction. The enzyme activity was expressed as ΔPH / 30 min. The Confidence limit in the plasma were 0.071 respectively and those of the erythrocytes were 0.148 respectively. The organophosphorus insecticides diazinon at concentration (0.25- 2 µM) in the reaction mixture significantly inhibited plasma (31~43%) and erythrocyte (52~67%) and dichlorvous at concentration (0.25 - 2 µM) in the reaction mixture significantly inhibited plasma (29~46%) and erythrocyte (55~68%) cholinesterase *in vitro* in a concentration-dependent. The insecticides cypermethrin at concentration (0.25 - 2 µM) in the reaction mixture significantly inhibited plasma lactic dehydrogenase by (19~22%). This experiments show activates of enzymes that affected by common insecticides used in Iraq.

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الخلاصة

استخدمت الطريقة الكهرومترية المحورة لقياس كفائة انزيم الكولين استراز في بلازما و كريات الدم الحمراء في الجمال حيث استخدمت عدة درجات حرارة لحديد الدرجة المثلى لنشاط الإنزيم وكانت بدرجة 37.5 مئوية من خلال احتساب التغير الناتج في باء هاء خليط التفاعل في 30 دقيقة حيث كان معمل الاختلاف في البلازما 0.017 وفي كريات الدم الحمراء 0.148، أدى تثبيط مزيج التفاعل بواسطة الدايازينون بتركيز (0.25 - 2 µM) الى تثبيط الانزيم في البلازما معنويا بنسبة (31−43%) وفي كريات الدم الحمراء (52−67%) وأدى تثبيط مزيج التفاعل بواسطة الدايكلورفس بتركيز (0.25 - 2 µM) الى تثبيط الانزيم في البلازما معنويا بنسبة (29−46%) وفي كريات الدم الحمراء (55−68%)، أدى تثبيط مزيج التفاعل بواسطة السايبرمثرين بتركيز (0.25 - 2 µM) الى تثبيط إنزيم اللاكتيك ديهيدروجينيز في البلازما معنويا بنسبة (19−22%).

Introduction

Determination of erythrocyte and plasma cholinesterase (ChE) activity is used to monitor exposure to organophosphate (OP) insecticides (1, 2, 3). Cypermethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade cypermethrin is a racemic mixture of 8 isomers (four cis and four trans isomers). Two stereoisomer is termed α-isomer of cypermethrin, which is believed to be the most active isomer, and is known as α-cypermethrin (α-CP) (4). Alfacypermethrin is extensively used as an ectoparasiticide in animals, and as insecticides in crop production and public health programme (4). Of the principle methods for measuring blood ChE activity is the electrometric method which is based on production of acetic acid that decreases the pH

of the reaction mixture (3, 5). The original electrometric method of Michel (6) is commonly used in man (3, 7). However, the method is not directly applicable to samples of different animal species (2, 3, 7). This is because of the inherent variations in blood ChE activities between different animal species (2, 3, 8, 9, 10, 11) and the special need for different buffer compositions, reaction temperature in incubation times and sample volumes (1, 2, 10, 11, 12) Various modifications of the electrometric method are available for measuring blood ChE activity in animals (1, 2, 3, 11, 13). These modifications include increasing sample volume, increasing or decreasing incubation time, increasing incubation temperature or using buffers of different compositions. A modified electrometric method was introduced for rapid measurement of erythrocyte and plasma ChE activities in sheep (12). It is characterized by its simplicity and one-step short incubation time 30 min (12). The method is based on measurement of the decrease in pH of the enzymatic reaction mixture as a result of hydrolysis of the substrate acetylcholine iodide or acetylthiocholine iodide and the production of acetic acid (12, 13). Some of the toxic actions of α-CP have been reported earlier (4, 14), but reports on tissue residue level and effects after repeated daily oral administration of α-CP on cytochrome P450, cytochrome b5, antioxidant status, blood biochemistry, and histology of some tissues in mammalians are not available. It has been recorded (8) that the vehicle has a great influence on the LD50, probably by influencing absorption. The oral LD50 values for rats were 79 mg/kg (4) But the report of LD50 value of α-CP for rats in presence of dimethylsulfoxide as a vehicle is not available.

Materials and Methods

All experiments complied with regulations addressing animal use, and proper attention has been given consideration towards the camel soused in the present study. the samples collect from 16 animals were apparently healthy and not exposed to any insecticide for at least one month before blood sampling. Blood samples were collected using EDTA's (AFCO, Jordan) test tubes. Plasma was separated from blood by centrifugation at 3.000 rpm (Hettich, Germany) for 15 min, and used freshly at following tests.

- **Electrometric procedure for measurement of plasma and erythrocyte ChE activities:** The modified electrometric method of Mohammad et.al. (12) Was used to measure blood ChE of the camel. For a typical assay, the reaction mixture in 10 ml beaker contained 3 ml distilled water 0 .2m l plasma or erythrocytes and 3ml of pH 8.1 buffer solution. The pH of the mixture (pH1) was measured with a glass electrode using a pH meter, and then 0.1m l of 7.5% aqueous solution of acetylcholine iodide (BDH, UK) was added to the mixture .The reaction mix flue was incubated at 37°C for 30 min. At the end of the incubation period, the pH of the reaction mixture (pH2) was measure the enzyme activity

The blank was without plasma or erythrocytes. The pH 8.1 buffer solution consisted of 1.237g sodium barbital (BDH, UK), 0.63 g potassium dihydrogen phosphate (Merck, Germany) and 35.07g sodium chloride (BDH, UK) dissolve in one liter of distilled water (12). Preliminary experiments using pooled plasma or erythrocyte samples indicated that an incubation time of 30 min after the addition of the substrate with a sample volume of 0.2 ml were suitable for measuring the ChE activity. The experiments described below were performed to standardize the present electrometric method in sheep, and to demonstrate its precision reproducibility, validity and efficiency in measuring enzyme inhibition, as well as other specification.

- ***In vitro* ChE activity by dichlovrvous and diazinon:** The method of inhibitor-ChE incubation (12, 15) was used to measure the in vitro of inhibition of plasma and erythrocyte ChE activity by dichlovrvous and diazinon.

The reaction mixture containing the insecticide were incubated at 37ºC for 10 min. thereafter, the residual ChE activity in the mixture was measure as before (15).

- ***In vitro* lactic dehydrogenase activity by cypermethrin:** This method bases on color metric method by uses of lactic dehydrogenase kit (Biolab, France) measured in computerized U.V. spectrophotometer (Labmed, USA) the kit prepare to zero conc. of cypermethrin as control and the residual cypermethrin activity in the mixture was measure as before.
- **Statistics:** When applicable the data were subjected to analysis of variance followed by the least significance difference test (16). Student's *t*-teat was used for the means of two groups and multiple *t*-tests (ANOVA) (16). The level of significance was at $p<0.05$, by using computerized program SPSS 10.

Results

- **Preliminary reference ChE activity:** A table 1 shows the normal ChE values, 95% confidence limit and related statistic for plasma and erythrocyte ChE activity of 8 camels. Preliminary reference values of the main cholinesterase activity (Δ pH/ 30 min**)** and confidence in the plasma were 0.071, respectively, and those of the erythrocyte were 0.148, respectively (Table 1). Erythrocyte ChE activity was significantly higher than that of the plasma.

Table (1) Preliminary reference cholinesterase activity (ΔPH/ 30 min) in the plasma and erythrocyte of camel

Sample	Plasma	Erythrocytes
Mean	0.218	0.389*
Standard error	0.044	0.208*
Standard deviation	0.5145	0.1739
Confidence	0.071	0.148

***Significant different from plasma cholinesterase activity p<0.05**

The Table 2 shows the optimum incubation temperature of reaction mixture that shown in (37.5ºC) the peak of ChE activity depends on animal body temperature.

Table (2) Effect of multiple degree of temperature to the peak of ChE activity in camel plasma and erythrocytes

Degree of temperature	Plasma ChE	Erythrocyte ChE
37.5ºC	0.44 ± 0.027	0.39 ± 0.012
36.5ºC	0.13 ± 0.006*	0.36 ± 0.003
35.5ºC	0.12 ± 0.004*	0.42 ± 0.013*

***Significant different from 37.5ºC cholinesterase activity p<0.05**

- *In vitro* ChE inhibition

1. The insecticide dichlorvous significantly and at a concentration-dependent inhibited plasma (29~46%) and erythrocyte (55~68%) ChE activity *in vitro* (Table 3).

Table (3) *In vitro* inhibition of camel plasma and erythrocyte cholinesterase (ChE) by dichlorvous (mean ± SE)

Inhibitor concentration µM	Plasma ChE		Erythrocyte ChE	
	ΔPH/ 30 min	% inhibition	ΔPH/ 30 min	% inhibition
0 µM	0.19 ± 0.13	0	0.22 ± 0.015	0
0.25 µM	0.06 ± 0.1	17	0.9 ± 0.022	14
0.5 µM	0.04 ± 0.01*	29	0.4 ± 0.05	31
1.0 µM	0.03 ± 0.021*	36	0.2 ± 0.6*	55
2.0 µM	0.05 ± 0.013*	46	0.09 ± 0.015*	68

***significantly different from the respective control (0 concentration) p<0.05.**

2. The insecticide diazinon significantly and at a concentration-dependent inhibited plasma (31~43%) and erythrocyte (52~67%) ChE activity *in vitro* (Table 4).

Table (4) *In vitro* inhibition of camel plasma and erythrocyte cholinesterase (ChE) by diazinon (mean ± SE)

Inhibitor concentration µM	Plasma ChE		Erythrocyte ChE	
	ΔPH/ 30 min	% inhibition	ΔPH/ 30 min	% inhibition
0 µM	0.16 ± 0.13	0	0.23 ± 0.005	0
0.25 µM	0.07 ± 0.06	15	0.33 ± 0.021	13
0.5 µM	0.05 ± 0.01*	31	0.2 ± 0.109	28
1.0 µM	0.05 ± 0.004*	34	0.2 ± 0.076*	62
2.0 µM	0.08 ± 0.054*	43	0.08 ± 0.07*	67

***significantly different from the respective control (0 concentration) p<0.05.**

3. The insecticide cypermethrin significantly and at a concentration-dependent inhibited plasma (19~22%) lactic dehydrogenase activity *in vitro* (Table 5).

Table (5) *In vitro* inhibition of camel of plasma lactic dehydrogenase by cypermethrin

Inhibitor concentration µM	Plasma ChE	
	Absorption	% inhibition
0 µM	8.68 ± 0.017	0
0.25 µM	2.02 ± 0.004	3
0.5 µM	1.26 ± 0.65	12
1.0 µM	0.94 ± 0.004*	19
2.0 µM	0.75 ± 0.003*	22

***significantly different from the respective control (0 concentration) p<0.05.**

Discussion

The methods available for measuring ChE activity have a wide range of variability and difficulties in reproducibility (2, 3, 7, 17) rather; the shortcomings of the original electrometric method are relative insensitivity, sample size and low through put (4). Several investigators advocated many modifications of the original electrometric method. These modifications included increasing the sample volume, increasing the reaction temperature, the use of different buffers and increasing or decreasing the incubation time (1, 2, 3, 11, 13). The present electrometric method described for measurement of blood ChE activities in goats depended mainly on the modification previously reported in goat(8, 12). The method has been applied successfully for the determination of blood or tissue ChE activities in other animals species such as chickens (12), rats (18), mice (13) as well as in man (8, 13). The method also decreases substantially handling of the reaction mixture as found in other electrometric methods (1, 2, 3, 13). In spite of the expected limitations of between laboratories comparisons (7, 17), the reference values or plasma and erythrocyte ChE activities of the camel, the described electrometric and colorimetric methods also presents for the first time blood ChE activity in this species. Sensitivity of the described method for detecting ChE inhibition caused by OP ChE inhibition should not be excluded from this in vitro system dewing the 30-min incubation time. In several temperature to given an optimum incubation temp. For the enzyme activity In addition. The described electrometric method in camel was also comparable to the original method from which it was derived in detecting low level of plasma ChE in camel (13). The inhibiton by cypermethrin in vitro effective to several enzymes present in plasma specially lactic dehydrogenase its necessary for metabolic aerobic path ways to convert of lactic acid to pyrovate (12).

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